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## A CELLULASE PREPARATION

## FIELD OF INVENTION

The present invention concerns a cellulase preparation comprising a single-component endoglucanase, a detergent additive comprising the cellulase preparation, a detergent composition containing the cellulase preparation as well as methods of treating cellulose-containing fabrics with the cellulase preparation.

## BACKGROUND OF THE INVENTION

10 It is well known in the art that repeated washing of cotton-containing fabrics generally causes a pronounced, unpleasant harshness in the fabric, and several methods for overcoming this problem have previously been suggested in the art. For example GB 1.368.599 of Unilever Ltd. teaches the  
15 use of cellulytic enzymes for reducing the harshness of cotton-containing fabrics. Also, US 4.435.307 (of Novo Industri A/S) teaches the use of a cellulytic enzyme derived from Humicola insolens as well as a fraction thereof, designated AC<sub>X</sub>I, as a harshness reducing detergent additive. Other uses  
20 of cellulytic enzymes mentioned in the art involve soil removal from and colour clarification of fabric (cf. for instance EP 220 016), providing increasing water absorption (JP-B-52-48236) and providing a localized variation in colour to give the treated fabrics a "stone-washed" appearance (EP  
25 307,564). Cellulytic enzymes may furthermore be used in the brewing industry for the degradation of  $\beta$ -glucans, in the baking industry for improving the properties of flour, in paper pulp processing for removing the non-crystalline parts of cellulose, thus increasing the proportion of crystalline  
30 cellulose in the pulp, and in animal feed for improving the digestibility of glucans.

The practical exploitation of cellulytic enzymes has, to some extent, been set back by the nature of the known

cellulase preparations which are often complex mixtures. It is difficult to optimise the production of multiple enzyme systems and thus to implement industrial cost-effective production of cellulytic enzymes, and their actual use has been  
5 hampered by difficulties arising from the need to apply rather large quantities of the cellulytic enzymes to achieve the desired effect on cellulosic fabrics.

The drawbacks of previously suggested cellulase preparations may be remedied by using preparations comprising  
10 a higher amount of endoglucanases. A cellulase preparation enriched in endoglucanase activity is disclosed in WO 89/00069.

#### SUMMARY OF THE INVENTION

A single endoglucanase component has now been iso-  
15 lated which exhibits favourable activity levels relative to cellulose-containing materials.

Accordingly, the present invention relates to a cellulase preparation consisting essentially of a homogenous endoglucanase component which is immunoreactive with a mono-  
20 clonal antibody raised against a partially purified  $\approx 43$  kD cellulase derived from Humicola insolens, DSM 1800.

The finding that this particular endoglucanase component of cellulase is advantageous for the treatment of cellulose-containing materials is of considerable practical significance: it permits a cost-effective production of the cellulase, e.g. by employing recombinant DNA techniques for producing the active component, and makes the actual effective application of the enzyme feasible in that a smaller quantity of the cellulase preparation is requested to produce the de-  
30 sired effect on cellulosic materials.

#### DETAILED DISCLOSURE OF THE INVENTION

The cellulase preparation of the invention is advantageously one in which the endoglucanase component exhibits a

CMC-endoase activity of at least about 50 CMC-endoase units per mg of total protein.

In the present context, the term "CMC-endoase activity" refers to the endoglucanase activity of the endoglucanase component in terms of its ability to degrade cellulose to glucose, cellobiose and triose, as determined by a viscosity decrease of a solution of carboxymethyl cellulose (CMC) after incubation with the cellulase preparation of the invention, as described in detail below.

Preferred cellulase preparations of the invention are those in which the endoglucanase component exhibits a CMC-endoase activity of at least about 60, in particular at least about 90, CMC-endoase units per mg of total protein. In particular, a preferred endoglucanase component exhibits a CMC-endoase activity of at least 100 CMC-endoase units per mg of total protein.

The CMC-endoase (endoglucanase) activity can be determined from the viscosity decrease of CMC, as follows:

A substrate solution is prepared, containing 35 g/l CMC (Hercules 7 LFD) in 0.1 M tris buffer at pH 9.0. The enzyme sample to be analyzed is dissolved in the same buffer.

10 ml substrate solution and 0.5 ml enzyme solution are mixed and transferred to a viscosimeter (e.g. Haake VT 181, NV sensor, 181 rpm), thermostated at 40°C.

Viscosity readings are taken as soon as possible after mixing and again 30 minutes later. The amount of enzyme that reduces the viscosity to one half under these conditions is defined as 1 unit of CMC-endoase activity.

SDS polyacrylamide gel electrophoresis (SDS-PAGE) and isoelectric focusing with marker proteins in a manner known to persons skilled in the art were used to determine the molecular weight and isoelectric point (pI), respectively, of the endoglucanase component in the cellulase preparation of the invention. In this way, the molecular weight of a specific endoglucanase component was determined to be  $\approx$  43 kD. The isoelectric point of this endoglucanase was determined to be about 5.1. The immunochemical characterization of

the endoglucanase was carried out substantially as described in WO 89/00069, establishing that the endoglucanase is immunoreactive with a monoclonal antibody raised against crude cellulase from Humicola insolens, DSM 1800. The cellobiohydrolase activity may be defined as the activity towards cellobiose p-nitrophenyl. The activity is determined as  $\mu$ mole nitrophenyl released per minute at 37°C and pH 7.0. The present endoglucanase component was found to have essentially no cellobiohydrolase activity.

10 A preferred endoglucanase component is one which has the following N-terminal amino acid sequence

Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Xaa Xaa Lys Pro Ser  
Xaa Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro Val Phe Ser  
Cys Asn Ala Asn Phe Gln Arg,

15 "Xaa" representing amino acids which have not yet been determined.

The endoglucanase component present in the cellulase preparation of the invention may preferably be one producible by species of Humicola such as Humicola insolens e.g strain  
20 DSM 1800, deposited on 1 October 1981 at the Deutsche Sammlung von Mikroorganismen, Mascheroder Weg 1B, D-3300 Braunschweig, FRG, in accordance with the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purposes of Patent Procedure (the  
25 Budapest Treaty).

The endoglucanase component in the cellulase preparation of the invention has been isolated by extensive purification procedures, i.a. involving immunoaffinity chromatography using a monoclonal antibody raised against a fraction  
30 of the crude cellulase from H. insolens, DSM 1800, and specific for a  $\approx$  43 kD endoglucanase present therein (cf. Example 1 below). This procedure has surprisingly resulted in the isolation of a  $\approx$  43 kD endoglucanase as a single component with unexpectedly favourable properties due to a surprisingly  
35 high endoglucanase activity.

For industrial production of the cellulase preparation according to the invention, however, it is preferred to employ recombinant DNA techniques or other techniques involving adjustments of fermentations or mutation of the microor-  
5 ganisms involved to ensure overproduction of the desired enzymatic activities. Such methods and techniques are known in the art and may readily be carried out by persons skilled in the art.

The endoglucanase component may thus be one which is  
10 producible by a method comprising cultivating a host cell transformed with a recombinant DNA vector which carries a DNA sequence encoding said endoglucanase component or a precursor of said endoglucanase component as well as DNA sequences encoding functions permitting the expression of the DNA se-  
15 quence encoding the endoglucanase component or precursor thereof, in a culture medium under conditions permitting the expression of the endoglucanase component or precursor thereof and recovering the endoglucanase component from the culture.

20 A DNA fragment encoding the endoglucanase component or a precursor thereof may, for instance, be isolated by establishing a cDNA or genomic library of a cellulase-producing microorganism, such as Humicola insolens, DSM 1800, and screening for positive clones by conventional procedures such  
25 as by hybridization to oligonucleotide probes synthesized on the basis of the full or partial amino acid sequence of the endoglucanase, or by selecting for clones expressing the appropriate enzyme activity (i.e. CMC-endoase activity as defined above), or by selecting for clones producing a protein  
30 which is reactive with an antibody against a native cellulase (endoglucanase) component.

Once selected, the DNA sequence may be inserted into a suitable replicable expression vector comprising appropriate promotor, operator and terminator sequences permitting  
35 the endoglucanase to be expressed in a particular host organism, as well as an origin of replication enabling the vector to replicate in the host organism in question.

The resulting expression vector may then be transformed into a suitable host cell, such as a fungal cell, e.g. a species of Aspergillus, most preferably Aspergillus oryzae or Aspergillus niger. Fungal cells may be transformed by a process involving protoplast formation and transformation of the protoplasts followed by regeneration of the cell wall in a manner known per se. The use of Aspergillus as a host microorganism is described in EP 238,023 (of Novo Industri A/S), the contents of which are hereby incorporated by reference. The host cell may also be a yeast cell, e.g. a strain of Saccharomyces cerevisiae.

Alternatively, the host organisms may be a bacterium, in particular strains of Streptomyces and Bacillus, and E. coli. The transformation of bacterial cells may be performed according to conventional methods, e.g. as described in Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor, 1989.

The screening of appropriate DNA sequences and construction of vectors may also be carried out by standard procedures, cf. Sambrook et al., op. cit.

The medium used to cultivate the transformed host cells may be any conventional medium suitable for growing the host cells in question. The expressed endoglucanase may conveniently be secreted into the culture medium and may be recovered therefrom by well-known procedures including separating the cells from the medium by centrifugation or filtration, precipitating proteinaceous components of the medium by means of a salt such as ammonium sulphate, followed by chromatographic procedures such as ion exchange chromatography, affinity chromatography, or the like.

By employing recombinant DNA techniques as indicated above, techniques of protein purification, techniques of fermentation and mutation or other techniques which are well known in the art, it is possible to provide endoglucanases of a high purity.

The cellulase preparation of the invention may conveniently be added to cellulose-containing fabrics together

with other detergent materials during soaking, washing or rinsing operations. Accordingly, in another aspect, the invention relates to a detergent additive comprising the cellulase preparation of the invention. The detergent additive may  
5 suitably be in the form of a non-dusting granulate, stabilized liquid or protected enzyme. Non-dusting granulates may be produced e.g. according to US 4,106,991 and 4,661,452 (both to Novo Industri A/S) and may optionally be coated by methods known in the art. Liquid enzyme preparations may,  
10 for instance, be stabilized by adding a polyol such as propylene glycol, a sugar or sugar alcohol, lactic acid or boric acid according to established methods. Other enzyme stabilizers are well known in the art. Protected enzymes may be prepared according to the method disclosed in EP 238,216.

15 The detergent additive may suitably contain 1 - 500, preferably 5 - 250, most preferably 10 - 100 mg of enzyme protein per gram of the additive. It will be understood that the detergent additive may further include one or more other enzymes, such as protease, lipase or amylase, conventionally  
20 included in detergent additives.

In a still further aspect, the invention relates to a detergent composition comprising the cellulase preparation of the invention.

Detergent compositions of the invention additionally  
25 comprise surfactants which may be of the anionic, non-ionic, cationic, amphoteric, or zwitterionic type as well as mixtures of these surfactant classes. Typical examples of anionic surfactants are linear alkyl benzene sulfonates (LAS), alpha olefin sulfonates (AOS), alcohol ethoxy sulfates (AES)  
30 and alkali metal salts of natural fatty acids.

Detergent compositions of the invention may contain other detergent ingredients known in the art as e.g. builders, bleaching agents, bleach activators, anti-corrosion agents, sequestering agents, anti soil-redeposition agents,  
35 perfumes, enzyme stabilizers, etc.

The detergent composition of the invention may be formulated in any convenient form, e.g. as a powder or



liquid. The enzyme may be stabilized in a liquid detergent by inclusion of enzyme stabilizers as indicated above. Usually, the pH of a solution of the detergent composition of the invention will be 7-12 and in some instances 7.0-10.5. Other  
5 detergent enzymes such as proteases, lipases or amylases may be included the detergent compositions of the invention, either separately or in a combined additive as described above.

The softening, soil removal and colour clarification  
10 effects obtainable by means of the cellulase preparation of the invention generally require a concentration of the cellulase preparation in the washing solution of 0.01 - 100, preferably 0.05 - 60, and most preferably 0.1 - 20 mg of enzyme protein per liter. The detergent composition of the invention  
15 is typically employed in concentrations of 0.5 - 20 g/l in the washing solution. In general, it is most convenient to add the detergent additive in amounts of 0.1 - 5% w/w or, preferably, in amounts of 0.2 - 2% of the detergent composition.

20 In a still further aspect, the present invention relates to a method of reducing the rate at which cellulose-containing fabrics become harsh or of reducing the harshness of cellulose-containing fabrics, the method comprising treating cellulose-containing fabrics with a cellulase preparation  
25 as described above. The present invention further relates to a method providing colour clarification of coloured cellulose-containing fabrics, the method comprising treating coloured cotton-containing fabrics with a cellulase preparation, and a method of providing a localized variation in  
30 colour of coloured cellulose-containing fabrics, the method comprising treating coloured cellulose-containing fabrics with a cellulase preparation. The methods of the invention may be carried out by treating cellulose-containing fabrics during washing. However, if desired, treatment of the fabrics  
35 may also be carried out during soaking or rinsing or simply by adding the cellulase preparation to water in which the fabrics are or will be immersed.

The present invention is described in further detail with reference to currently preferred embodiments in the following examples which are not intended to limit the scope of the invention in any way.

## 5 EXAMPLES

### Example 1

#### Isolation of a $\approx$ 43 kD endoglucanase from *Humicola insolens*

1. Preparation of a monoclonal antibody reactive with an endoglucanase in Celluzyme<sup>TM</sup>.

10 Cellulase was produced by cultivating *Humicola insolens* DSM 1800, as described in US 4,435,307, Example 6. The crude cellulase was recovered from the culture broth by filtration on diatomaceous earth, ultrafiltration and freeze-drying of the retentate, cf. Examples 1 and 6 of US  
15 4,435,307.

The crude cellulase was purified as described in WO 89/09259, resulting in the fraction F1P1C2 which was used for the immunization of mice. The immunization was carried out 5 times at bi-weekly intervals, each time using 25  $\mu$ g protein  
20 including Freund's Adjuvant.

Hybridoma cell lines were established as described in Ed Harlow and David Lane, Antibodies. A Laboratory Manual, Cold Spring Harbor Laboratory 1988. The procedure may briefly be described as follows:

25 After bleeding the mouse and showing that the mouse serum reacts with proteins present in the F1P1C2 fraction, the spleen was removed and homogenized and then mixed with PEG and Fox-river myeloma cells from Hyclone, Utah, USA.

The hybridomas was selected according to the established HAT screening procedure.  
30

The recloned hybridoma cell lines were stabilized. The antibodies produced by these cell lines were screened and selected for belonging to the IgG1 subclass using a commer-

cial mouse monoclonal typing kit from Serotec, Oxford, England. Positive antibodies were then screened for reactivity with F1P1C2 in a conventional ELISA, resulting in the selection of F4, F15 and F41 as they were all very good in ELISA response but were found to have different response in immunoblotting using Celluzyme (crude *H. insolens*, DSM 1800, cellulase) in SDS-PAGE followed by Western Blot, indicating that they recognized different epitopes.

The three antibodies were produced in large quantities in the ascites fluid of CRBF<sub>1</sub> mice. The mouse gamma-globulin was purified from ascites fluid by protein A purification using protein A coupled to Sepharose (Kem.En.Tek., Copenhagen, Denmark).

The different monoclonal gammaglobulins were tested for response in a sandwich ELISA using each monoclonal antibody as the catching antibody, various HPLC fractions of Celluzyme as the antigen, and a rabbit antibody raised against endoglucanase B from Celluzyme as the detection antibody.

To visualize binding in the ELISA, a porcine antibody against rabbit IgG covalently coupled to peroxidase from Dakopatts (Copenhagen, Denmark) and was visualized with OPD(1,2-phenylenediamine, dihydrochloride)/H<sub>2</sub>O<sub>2</sub>.

The highest ELISA response was obtained with the monoclonal antibody F41 which was therefore used in the immunoadfinity purification steps.

The purified mouse gammaglobulin F41 was coupled to 43 g of CNBr-activated Sepharose 4B as described by the manufacturer (Pharmacia, Sweden) followed by washing.

## 2. Immunoaffinity purification of a $\approx$ 43 kD endoglucanase from Celluzyme

Celluzyme was diluted to 3% dry matter, and the pH was adjusted to 3.5 in 15 min. at 4 °C. The precipitate was removed by filtration after adjusting the pH to 7.5. Then sodium sulphate was added to precipitate the active enzyme and this was done at 40°C (260 gram per kg at pH 5.5). The precipitate was solubilized with water and filtrated. The acid

treatment was repeated. Finally, the product was filtrated and concentrated by ultrafiltration using a polyvinylsulphonate membrane with a 10.000 Mw cut-off.

The cellulase product was then diluted to 3% dry matter, adjusting the pH to 9.0, and subjected to anion exchange chromatography on a DEAE-Sepharose column as recommended by the manufacturer (Pharmacia, Sweden).

The protease-free cellulase product was applied on the F 41 gammaglobulin-coupled Sepharose column described above at pH 8.0 in sodium phoshate buffer.

After application the column was washed with the same buffer containing 0.5 M sodium chloride. The column was then washed with 0.1 M sodium acetate buffer containing 0.5 M sodium chloride, pH 4.5, after which the column was washed in 5 mM sodium acetate buffer, pH 4.5. Finally, the 43 kD endoglucanase was eluted with 0.1 M citric acid.

Total yield: 25 mg with an endoglucanase activity of 1563 CMC-endoase units.

The eluted protein migrates as a single band in SDS-PAGE with an apparent MW of 43 kD and a pI after isoelectric focusing of about 5.0 to 5.2.

Inactive protein was removed by reverse phase purification.

Inactive and active protein was separated by HPLC using a gradient of 2-propanol. Inactive protein elutes at about 25% 2-propanol and the active 43 kD endoglucanase elutes at 30% 2-propanol, the active endoglucanase being detectable by a CMC-Congo Red clearing zone.

In this way, a total of 0.78 mg active protein was recovered with 122 CMC endoase units. This procedure was repeated 30 times.

The 43 kD endoglucanase was recovered by first freeze-drying to remove the TFA and propanol and then solubilizing in phosphate buffer.

The endoglucanase activity of the purified material was 156 CMC-endoase units per mg protein and the total yield including freze-drying was 65% of the endoglucanase activity.

### 3. Characterization of 43 kD endoglucanase:

Amino acid composition: Using total hydrolysis, the following composition was obtained after amino acid analysis:

|    |         |            |
|----|---------|------------|
|    | Asp/Asn | 43         |
| 5  | Thr     | 33         |
|    | Ser     | 42         |
|    | Glu/Gln | 26         |
|    | Pro     | 28         |
|    | Gly     | 43         |
| 10 | Ala     | 31         |
|    | Cys     | 26 minimum |
|    | Val     | 19         |
|    | Met     | 3          |
|    | Ile     | 9          |
| 15 | Leu     | 11         |
|    | Tyr     | 8          |
|    | Phe     | 20         |
|    | Lys     | 12         |
|    | His     | 3          |
| 20 | Trp     | 9          |
|    | Arg     | 16         |

Mw of unglycosylated protein was estimated to be 40 kD based on the amino acid composition. The extinction coefficient per mole was estimated as follows:

|    |                          |
|----|--------------------------|
| 25 | Tryptophane 9 times 5690 |
|    | Tyrosine 8 times 1280    |
|    | Cysteins 26 times 120    |
|    | total 64570 per mole.    |

Extinction coefficients are 1.61 at 280 nm corresponding to 1  
30 mg protein per ml. (Reference: S.C.Gill and P. Hippel, Anal. Biochemistry 182, 312-326 (1989).)

The N-terminal amino acid sequence was determined on an Applied Biosystems 475A Protein Sequenator using Edman de-

gradation. Only one sequence indicated the purity of the protein. The N-terminal sequence is shown in the conventional three-letter code:

Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Xaa Xaa Lys Pro Ser  
5 Xaa Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro Val Phe Ser  
Cys Asn Ala Asn Phe Gln Arg,

"Xaa" representing amino acids which have not yet been determined.

The amino acids not yet determined could be either  
10 Cys or glucosylated species.

#### Enzyme properties:

The enzyme is stable between pH 3 and 9.5.

The enzyme does not degrade highly crystalline cellulose or the substrate cellobiose  $\beta$ -p-nitrophenyl, (Cello-  
15 biohydrolase substrate).

The enzyme is active between pH 6.0 and 10.0 with a maximum activity at about 50°C.

#### Example 2

The  $\approx$  43 kD endoglucanase (a mixture of 30 purification runs) was compared in a colour clarification test with the Celluzyme preparation described in US 4,435,307, Example 6.

Old worn black cotton swatches are used as the test material. The clarification test is made in a Terg-O-tometer  
25 making three repeated washes. Between each wash the swatches are dried overnight.

#### Conditions:

2 g/l of liquid detergent at 40°C in 30 min. and a water hardness of 9°dH. The swatch size is 10x15 cm, and  
30 there are two swatches in each beaker.

The composition of the detergent was as follows:

14

10% anionic surfactant (Nansa 1169/p)

15% non-ionic surfactant (Berol 160)

10% ethanol

5% triethanol amine

5 60% water

pH adjusted to 8.0 with HCl.

Dosage:

The two enzymes are dosed in 63 and 125 CMC-endoase units/l.

10 Results:

The results were evaluted by a panel of 22 persons who rated the swatches on a scale from 1 to 7 points. The higher the score, the more colour clarification obtained.

| Enzyme    | CMC-endoase/l | Protein mg/l | PSU*                   |
|-----------|---------------|--------------|------------------------|
| No enzyme |               |              | 1.4 ± 1.0              |
| Celluzyme | 63<br>125     | 14<br>28     | 5.8 ± 1.0<br>6.1 ± 1.0 |
| Invention | 63<br>125     | 0.4<br>0.8   | 4.6 ± 0.9<br>6.2 ± 0.8 |

\* PSU = Panel Score Units

The ≈ 43 kD endoglucanase is shown to have an about 30 times better performance than the prior art Celluzyme, and an about 6 times better performance than the cellulase preparation according to WO 89/09259.

## CLAIMS

1. A cellulase preparation consisting essentially of a homogenous endoglucanase component which is immunoreactive with a monoclonal antibody raised against a partially purified  $\approx$  43 kD cellulase derived from Humicola insolens, DSM 1800.

2. A cellulase preparation according to claim 1, wherein the endoglucanase component has an endoglucanase activity of at least 50 CMC-endoase units/mg of protein.

3. A cellulase preparation according to claim 2, wherein the endoglucanase component has an endoglucanase activity of at least 60 CMC-endoase units/mg of total protein, in particular at least 90 CMC-endoase units/mg of total protein, and preferably at least 100 CMC-endoase units/mg of total protein.

4. A cellulase preparation according to claim 1, wherein the endoglucanase component has essentially no cellobiohydrolase activity.

5. A cellulase preparation according to claim 1, wherein the endoglucanase component has the following N-terminal amino acid sequence

Ala Asp Gly Arg Ser Thr Arg Tyr Trp Asp Xaa Xaa Lys Pro Ser  
Xaa Gly Trp Ala Lys Lys Ala Pro Val Asn Gln Pro Val Phe Ser  
Cys Asn Ala Asn Phe Gln Arg,

"Xaa" representing amino acids which have not yet been determined.

6. A cellulase preparation according to any of claims 1 - 5, wherein the endoglucanase component has an isoelectric point of about 5.1.

7. A cellulase preparation according to any of claims 1 - 6, wherein the endoglucanase component is one producible by a species of Humicola, e.g. Humicola insolens.

8. A cellulase preparation according to any of claims 1 - 7, wherein said endoglucanase component is pro-



ducible by a method comprising cultivating a host cell transformed with a recombinant DNA vector carrying a DNA sequence encoding said endoglucanase component or a precursor of said endoglucanase component as well as DNA sequences encoding  
5 functions permitting the expression of the DNA sequence encoding the endoglucanase component, or a precursor thereof, in a culture medium under conditions permitting the expression of the endoglucanase component or precursor thereof and recovering the endoglucanase component from the culture.

10 9. A cellulase preparation according to claim 8, wherein the host cell is a strain of a fungus such as Aspergillus, preferably Aspergillus oryzae or Aspergillus niger, or a yeast cell, e.g. a strain of Saccharomyces cerevisiae.

15 10. A cellulase preparation according to claim 8, wherein the host cell is a strain of a bacterium, e.g. Bacillus, Streptomyces or E. coli.

20 11. A detergent additive containing a cellulase preparation according to any of claims 1 - 10, preferably in the form of a non-dusting granulate, stabilized liquid or protected enzyme.

12. A detergent additive according to claim 8 which contains 1 - 500, preferably 5 - 250, most preferably 10-100, mg of enzyme protein per gram of the additive.

25 13. A detergent additive according to claim 11 which additionally comprises another enzyme such as a protease, lipase and/or amylase.

14. A detergent composition comprising a cellulase preparation according to any of claims 1 - 10.

30 15. A detergent composition according to claim 14, which additionally comprises another enzyme such as a protease, lipase and/or amylase.

16. A detergent composition according to claim 14, wherein the cellulase preparation is present in a concentration corresponding to 0.01 - 100, preferably 0.05 - 60, and  
35 most preferably 0.1 - 20, mg of enzyme protein per liter washing solution.

17. A detergent composition comprising a detergent additive according to any of claims 11- 13.

18. A method of reducing the rate at which cellulose-containing fabrics become harsh or of reducing the harshness of cellulose-containing fabrics, the method comprising treating cellulose-containing fabrics with a cellulase preparation according to any of claims 1 - 10.

19. A method of providing colour clarification of coloured cellulose-containing fabrics, the method comprising treating coloured cotton-containing fabrics with a cellulase preparation according to any of claims 1 - 10.

20. A method of providing a localized variation in colour of coloured cellulose-containing fabrics, the method comprising treating coloured cotton-containing fabrics with a cellulase preparation according to any of claims 1 - 10.

21. A method according to any of claims 18, 19 or 20, wherein the treatment of the fabrics with the cellulase preparation is carried out during soaking, washing or rinsing of the fabrics.